



Marked-Up Copy of Amended Claims

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15. (Amended) A cultured tissue construct having at least two layers, comprising:

(a) a first layer of cultured fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer;  
and,

(c) The bilayered cultured tissue construct of claim 9, further comprising a third layer of cells disposed on the second layer of epithelial cells.

16. A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) fibronectin,

(iv) tenascin; and,

(v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is a multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

17. (Amended) The construct of any of claims ~~4, 8, 9, 15~~ and 16, wherein the cultured fibroblast cells are genetically modified to produce extracellular matrix components.

18. The construct of claim 17, wherein the cultured fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

28. (Amended) A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting a cultured tissue construct of any of claims ~~4, 8, 9, 15~~ or 16 into a patient in need of treatment thereof.

29. A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components under culturing conditions in a second culture medium; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

- (i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;
- (ii) tenascin; and,
- (iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

30. (Amended) The construct of any of claims 415-18, wherein the construct is cohesive in having physical unitary integrity and tissue-like handling properties.